CLAIMS

We claim:

1. A method for making transcription product corresponding to a target nucleic acid sequence, the method comprising:

(a) obtaining an RNA polymerase that can transcribe RNA using a single-stranded promoter;

(b) obtaining a single-stranded DNA wherein the single-stranded DNA comprises a target nucleic sequence that is present in or complementary to at least a portion of a target nucleic acid in a sample;

(c) obtaining a single-stranded transcription substrate by operably joining to the single-stranded DNA a single-stranded polynucleotide comprising a promoter sequence that binds the RNA polymerase;

(d) obtaining nucleoside triphosphates (NTPs) that are substrates for the RNA polymerase and that are complementary to canonical nucleic acid bases;

(e) admixing the RNA polymerase, the single-stranded transcription substrate and the NTPs; and

(f) incubating the RNA polymerase and the single-stranded transcription substrate under conditions effective to allow synthesis of transcription product.

2. A method for making transcription product corresponding to a target nucleic acid sequence, the method comprising:

(a) obtaining an RNA polymerase that can transcribe RNA using a single-stranded promoter;

(b) obtaining a single-stranded DNA wherein the single-stranded DNA comprises a target nucleic acid sequence that is present in or complementary to at least a portion of a target nucleic acid in a sample;

(c) obtaining a single-stranded transcription substrate by operably joining to the single-stranded DNA a single-stranded polynucleotide comprising a promoter sequence that binds the RNA polymerase;

(d) admixing the RNA polymerase and the single-stranded transcription substrate; and

(e) incubating the RNA polymerase and the single-stranded ssDNA transcription substrate under conditions effective to allow synthesis of transcription product.

3. A method for obtaining additional rounds of synthesis of transcription product corresponding to a target nucleic acid sequence, the method comprising:

(a) obtaining an RNA polymerase that can transcribe RNA using a single-stranded promoter;

(b) obtaining a first transcription product by transcription of a first single-stranded transcription substrate comprising a polynucleotide corresponding to a target nucleic acid sequence;

(c) obtaining a reverse transcriptase;

(d) reverse transcribing the first single-stranded transcription product;

(e) obtaining first-strand cDNA complementary to the first single-stranded transcription product;

(f) obtaining a second single-stranded transcription substrate by operably joining to the first-strand cDNA a single-stranded polynucleotide comprising a promoter sequence that binds the RNA polymerase;

(g) admixing the RNA polymerase and the second single-stranded transcription substrate; and

(h) incubating the RNA polymerase and the second single-stranded transcription substrate under conditions effective to allow synthesis of a second transcription product.

4. The method of claim 1 wherein the RNA polymerase comprises a region encoding a polypeptide having an amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:15.

5. The method of claim 1 wherein the RNA polymerase comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:14.

6. The method of claim 1 wherein the promoter is an N4 vRNAP promoter set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

7. The method of claim 1 wherein the promoter is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

8. The method of claim 1 wherein the RNA polymerase is E. coli RNAP and the promoter is a single-stranded pseudopromoter for E.coli RNAP.

9. The method of claim 1 wherein the RNA polymerase is a T7-type RNAP and the promoter is a cognate single-stranded pseudopromoter for the T7-type RNAP.

10. The method of claim 1 wherein the RNA polymerase is T7 RNAP and the promoter is a single-stranded pseudopromoter for T7 RNAP.

11. The method of claim 1 wherein the RNA polymerase is T3 RNAP and the promoter is a single-stranded pseudopromoter for T3 RNAP.

12. The method of claim 1 wherein the RNA polymerase is SP6 RNAP and the promoter is a single-stranded pseudopromoter for SP6 RNAP.

13. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence comprising an RNA target nucleic acid.

14. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence comprising an mRNA target nucleic acid.

15. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence comprising an mRNA target nucleic acid that is full-length.

16. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence comprising mRNA target nucleic acid corresponding to substantially all mRNA in the sample.

17. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence comprising a DNA target nucleic acid.

18. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence comprising a DNA target nucleic acid that is a product of an amplification reaction.

19. The method of claim 1 wherein the amplification reaction is selected from the group consisting of PCR, RT-PCR, NASBA, TMA, 3SR, LCR, LLA, SDA, RCA, Multiple Displacement Amplification, ICAN™, UCAN™, Loop-AMP, SPIA™ and Ribo-SPIA™.

20. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence that is obtained by primer extension of a larger DNA target nucleic acid.

21. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence that is obtained by reverse transcriptase primer extension of at least one mRNA target nucleic acid.

22. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence that is obtained by reverse transcriptase primer extension of substantially all mRNA target nucleic acid in a sample.

23. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence that has a tail sequence comprising at least two nucleotides.

24. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence that has a tail sequence comprising of dCMP nucleotides.

25. The method of claim 1 wherein the single-stranded DNA comprises a target nucleic acid sequence that has a tail sequence between two to ten nucleotides.

26. The method of claim 1 wherein the ssDNA transcription substrate of step (c) is obtained using a promoter splice template oligo.

27. The method of claim 26 wherein the single-stranded DNA is obtained by reverse transcription of a transcription product.

28. The method of claim 1 wherein the single-stranded DNA in step (c) is obtained by reverse transcription of a transcription product prepared using the method of claim 26.

29. The method of claim 1, wherein the ssDNA transcription substrate is obtained by using a promoter ligation oligo and a ligation splint.

30. The method of claim 1, wherein the single-stranded DNA is obtained by reverse transcription of a transcription product prepared using a promoter ligation oligo and a ligation splint.

31. The method of claim 1 wherein a single-stranded transcription substrate is obtained by DNA polymerase-catalyzed primer extension of a promoter primer using the target nucleic acid in the sample as a template, followed by ligation of the 5'-end of the primer-extended promoter primer to the 3'-end primer extension, comprising the single-stranded DNA comprising the target nucleic acid sequence, thereby operably joining the promoter to the target nucleic acid sequence to form a circular single-stranded transcription substrate.

32. The method of claim 31 wherein the target nucleic acid in the sample comprises RNA and the DNA polymerase used for primer extension is an enzyme with reverse transcriptase activity.

33. The method of claim 31 wherein the target nucleic acid in the sample comprises mRNA.

34. The method of claim 31 further comprising the DNA polymerase used for primer extension is an enzyme with reverse transcriptase activity.

35. The method of claim 31 wherein the single-stranded DNA comprising a target nucleic acid sequence is obtained by reverse transcription of a transcription product prepared using the method of claim 1.

36. The method of claim 1 wherein a linear single-stranded transcription substrate is obtained by cleaving a circular single-stranded transcription substrate obtained using the method of claim 31 at a site that is 3'-of the promoter sequence and 5'-of the target nucleic acid sequence.

37. The method of claim 1 wherein the single-stranded DNA comprising a target nucleic acid sequence is obtained by reverse transcription of a transcription product prepared using the method of claim 31.

38. The method of claim 1, wherein at least one of the NTPs comprises a 2'-amino-deoxynucleoside triphosphate.

39. The method of claim 1, wherein at least one of the NTPs comprises a 2'-amino-dCTP.

40. The method of claim 1, wherein at least one of the NTPs comprises a 2'-fluoro-deoxynucleoside triphosphate.

41. The method of claim 1, wherein at least one of the NTPs comprises a 2'-fluoro-dCTP.

42. The method of claim 1, wherein at least one of the NTPs comprises a 2'-fluoro-dUTP.

43. The method of claim 1, wherein at least one of the NTPs comprises a 2'-azido-deoxynucleoside triphosphate.

44. The method of claim 1, wherein at least one of the NTPs comprises a 2'-azido-dCTP.

45. The method of claim 1 wherein the NTPs are complementary to canonical nucleic acid bases.

46. A kit for performing the method of claim 1 wherein the kit comprises an RNA polymerase that can use a single-stranded promoter for transcription of RNA and a promoter splice template oligo.

47. The kit of claim 46 wherein the RNA polymerase comprises a region encoding a polypeptide having an amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:15.

48. The kit of claim 47 wherein the promoter is an N4 vRNAP promoter set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

49. The kit of claim 47 wherein the promoter is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

50. The kit of claim 46 wherein the RNA polymerase comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:14.

51. The kit of claim 46 wherein the RNA polymerase comprises E. coli RNAP and the promoter splice template comprises a single-stranded pseudopromoter for E.coli RNAP.

52. The kit of claim 46 wherein the RNA polymerase comprises T7-type RNAP and the promoter splice template oligo comprises a single-stranded pseudopromoter for the T7-type RNAP.

53. The kit of claim 46 wherein the RNA polymerase comprises T7 RNAP and the promoter splice template oligo comprises a single-stranded pseudopromoter for T7 RNAP.

54. The kit of claim 46 wherein the RNA polymerase comprises T3 RNAP and the promoter splice template oligo comprises a single-stranded pseudopromoter for T3 RNAP.

55. The kit of claim 46 wherein the RNA polymerase comprises SP6 RNAP and the promoter splice template oligo comprises a single-stranded pseudopromoter for SP6 RNAP.

56. A kit for performing the method of claim 1 wherein the kit comprises an RNA polymerase that can use a single-stranded promoter for transcription of RNA and a promoter ligation oligo.

57. The kit of claim 56 wherein the RNA polymerase comprises a region encoding a polypeptide having an amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:15.

58. The kit of claim 57 wherein the promoter is an N4 vRNAP promoter set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

59. The kit of claim 57 wherein the promoter is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

60. The kit of claim 56 wherein the RNA polymerase comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:14.

61. The kit of claim 56 wherein the promoter is an N4 vRNAP promoter set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

62. The kit of claim 56 wherein the promoter is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

63. The kit of claim 56 wherein the RNA polymerase comprises E. coli RNAP and the promoter ligation oligo comprises a single-stranded pseudopromoter for E.coli RNAP.

64. The kit of claim 56 wherein the RNA polymerase comprises T7-type RNAP and the promoter ligation oligo comprises a single-stranded pseudopromoter for the T7-type RNAP.

65. The kit of claim 56 wherein the RNA polymerase comprises T7 RNAP and the promoter ligation oligo comprises a single-stranded pseudopromoter for T7 RNAP.

66. The kit of claim 56 wherein the RNA polymerase comprises T3 RNAP and the promoter ligation oligo comprises a single-stranded pseudopromoter for T3 RNAP.

67. The kit of claim 56 wherein the RNA polymerase comprises SP6 RNAP and the promoter ligation oligo comprises a single-stranded pseudopromoter for SP6 RNAP

68. A kit for performing the method of claim 1 wherein the kit comprises an RNA polymerase that can use a single-stranded promoter for transcription of RNA and a promoter primer.

69. The kit of claim 68 wherein the RNA polymerase comprises a region encoding a polypeptide having an amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:7, SEQ ID NO:8 or SEQ ID NO:15.

70. The kit of claim 69 wherein the promoter is an N4 vRNAP promoter set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

71. The kit of claim 69 wherein the promoter is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

72. The kit of claim 68 wherein the RNA polymerase comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:14.

73. The kit of claim 68 wherein the promoter is an N4 vRNAP promoter set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

74. The kit of claim 68 wherein the promoter is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

75. The kit of claim 68 wherein the RNA polymerase comprises E. coli RNAP and the promoter primer comprises a single-stranded pseudopromoter for E.coli RNAP.

76. The kit of claim 68 wherein the RNA polymerase comprises T7-type RNAP and the promoter primer comprises a single-stranded pseudopromoter for the T7-type RNAP.

77. The kit of claim 68 wherein the RNA polymerase comprises T7 RNAP and the promoter primer comprises a single-stranded pseudopromoter for T7 RNAP.

78. The kit of claim 68 wherein the RNA polymerase comprises T3 RNAP and the promoter primer comprises a single-stranded pseudopromoter for T3 RNAP.

79. The kit of claim 68 wherein the RNA polymerase comprises SP6 RNAP and the promoter primer comprises a single-stranded pseudopromoter for SP6 RNAP

80. The method of claim 1 wherein the target nucleic acid sequence comprises a 3'-portion that encodes a first sequence, a 5'-portion that encodes a second sequence that is complementary to the first sequence, and a middle portion that joins the 3'portion and the 5'portion, wherein the middle portion comprises a sequence that is not complementary to either the 3'-portion or the 5'portion and wherein the transcription product comprises a hairpin RNA.

81. The method of claim 80, wherein the hairpin RNA corresponds to a target nucleic acid sequence in a target nucleic acid comprising an mRNA.

82. The method of claim 80 , wherein the hairpin RNA has RNA interference activity in a cell that synthesizes an mRNA target nucleic acid comprising the target nucleic acid sequence.

83. The method of claim 80 wherein the hairpin RNA comprises siRNA.

84. The method of claim 80 wherein the hairpin RNA comprises at least one modified nucleoside triphoshate.

85. The method of claim 84 wherein the modified nucleoside triphosphate is selected from the group consisting of 2'-amino-deoxynucleoside triphosphate, 2'-amino-dCTP, 2'-fluoro-deoxynucleoside triphosphate, 2'-fluoro-dCTP, 2'-fluoro-dUTP, 2'-azido-deoxynucleoside triphosphate, and 2'-azido-dCTP.

86. The method of claim 80 wherein the hairpin RNA is at least 40 nucleotides in length.

87. The method of claim 80 wherein the hairpin RNA is at least 100 nucleotides in length.

88. The method of claim 80 wherein the hairpin RNA is made with an RNA polymerase that can use a single-stranded promoter for transcription of RNA

89. The method of claim 88, wherein the RNA polymerase comprises a region encoding a polypeptide having an amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:15.

90. The method of claim 88, wherein the single-stranded promoter is an N4 vRNAP promoter set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

91. The method of claim 88 wherein the single-stranded promoter is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

92. The method of claim 88, wherein the RNA polymerase comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:14.

93. The method of claim 88 wherein the RNA polymerase comprises E. coli RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for E. coli RNAP.

94. The method of claim 88 wherein the RNA polymerase comprises T7-type RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for the T7-type RNAP.

95. The method of claim 88 wherein the RNA polymerase comprises T7 RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for T7 RNAP.

96. The method of claim 88, wherein the RNA polymerase comprises T3 RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for T3 RNAP.

97. The method of claim 88 wherein the RNA polymerase comprises SP6 RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for SP6 RNAP.

98. The method of claim 80 wherein the hairpin RNA is made in vitro.

99. The method of claim 80 wherein the hairpin RNA is made in vivo.

100. A method for attenuating expression of a target gene in a cell comprising introducing the hairpin RNA of claim 80 into the cell.

101. The method of claim 100 wherein the expression of a target gene in the cell is attenuated in vitro.

102. The method of claim 100 wherein the expression of the target gene in the cell is attenuated in vivo.

103. The method of claim 100, wherein the cell comprises a mammalian cell.

104. The method of claim 103, wherein the mammalian cell comprises a human cell.

105. A hairpin RNA made by the method of claim 80.

106. A cell comprising a hairpin RNA made by the method of claim 80.

107. A kit for making the hairpin of claim 80, the kit comprising an RNA polymerase that can use a single-stranded promoter for transcription of RNA and an oligonucleotide comprising a sequence corresponding to a single-stranded promoter sequence.

108. A method of cloning a target nucleic acid, the method comprising:

a) obtaining a single-stranded DNA wherein the single-stranded DNA comprises a target nucleic acid sequence that is present in or complementary to the target nucleic acid;

b) joining to the single-stranded DNA a single-stranded polynucleotide comprising a single-stranded origin of replication and a marker gene;

c) making a circular ssDNA molecule by covalently joining the 3'-end and the 5'-end of the product of step (b);

d) transforming the circular ssDNA molecule into a host cell, in which the marker gene is expressible, wherein the host cell is capable of replicating the circular ssDNA molecule; and

e) growing the host cell under conditions that support the expression of the marker gene.

109. The method of claim 108, wherein the target nucleic acid comprises an RNA target nucleic acid.

110. The method of claim 108, wherein the target nucleic acid comprises an mRNA target nucleic acid.

111. The method of claim 108, wherein the target nucleic acid sequence comprises an mRNA target nucleic acid that is full-length.

112. The method of claim 108, wherein the target nucleic acid comprises an mRNA target nucleic acid corresponding to substantially all mRNA within a sample.

113. The method of claim 108, wherein the target nucleic acid comprises a DNA target nucleic acid.

114. The method of claim 108, wherein the target nucleic acid comprises a target nucleic acid that is a product of an amplification reaction.

115. The method of claim 114 wherein the amplification reaction is selected from the group consisting of PCR, RT-PCR, NASBA, TMA, 3SR, LCR, LLA, SDA, RCA, Multiple Displacement Amplification, ICAN™, UCAN™, Loop-AMP, SPIA™ and Ribo-SPIA™.

116. The method of claim 108 wherein the target nucleic is obtained by primer extension of a larger DNA target nucleic acid.

117. The method of claim 108 wherein the target nucleic acid is obtained by reverse transcriptase primer extension of at least one mRNA target nucleic acid.

118. The method of claim 108 wherein the target nucleic acid sequence is obtained by reverse transcriptase primer extension of substantially all mRNA target nucleic acids in a sample.

119. The method of claim 108, wherein the single-stranded polynucleotide comprising a single-stranded origin of replication and a marker gene are joined to the single-stranded DNA by using a promoter splice template oligo.

120. The method of claim 108, wherein the single-stranded polynucleotide comprising a single-stranded origin of replication and a marker gene are joined to the single-stranded DNA by using a promoter ligation oligo.

121. The method of claim 108, wherein the single-stranded origin of replication comprises an M13 origin of replication.

122. The method of claim 108, wherein the marker gene comprises an antibiotic-resistance gene.

123. The method of claim 108, wherein the marker gene comprises a beta-galactosidase gene.

124. The method of claim 108, wherein the single stranded polynucleotide comprises a transposon recognition sequence.

125. The method of claim 108, wherein the single-stranded polynucleotide comprises a site that can be recognized by a recombinase.

126. The method of claim 108, wherein the circular ssDNA molecule is made by using a ligase that catalyzes non-homologous intramolecular ligation.

127. The method of claim 126, wherein the ligase is ThermoPhage™ RNA Ligase II.

128. The method of claim 108, wherein the circular DNA molecule is made by DNA polymerase-catalyzed primer extension of a primer using the target nucleic acid as a template, followed by ligation of the 3'-end of the primer extension product to the 5'-end of the primer extension product, wherein the primer comprises a single-stranded origin of replication and a marker gene.

129. A method of constructing a nucleic acid library comprising clones of substantially all nucleic acids within a sample by using the method of claim 108.

130. The method of claim 108, wherein the single-stranded polynucleotide comprises a single-stranded promoter that binds a RNA polymerase that can transcribe RNA using a promoter that is single-stranded.

131. The method of claim 130, wherein the RNA polymerase comprises a region encoding a polypeptide having an amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:15.

132. The method of claim 130, wherein the single-stranded promoter is an N4 vRNAP promoter set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

133. The method of claim 130 wherein the single-stranded promoter is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

134. The method of claim 130, wherein the RNA polymerase comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:14.

135. The method of claim 130 wherein the RNA polymerase comprises E. coli RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for E.coli RNAP.

136. The method of claim 130 wherein the RNA polymerase comprises T7-type RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for the T7-type RNAP.

137. The method of claim 130 wherein the RNA polymerase comprises T7 RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for T7 RNAP.

138. The method of claim 130, wherein the RNA polymerase comprises T3 RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for T3 RNAP.

139. The method of claim 130 wherein the RNA polymerase comprises SP6 RNAP and the single-stranded promoter comprises a single-stranded pseudopromoter for SP6 RNAP.

140. The method of claim 108, wherein the single-stranded polynucleotide comprises a single-stranded promoter that binds a RNA polymerase that can transcribe RNA using a promoter that is single-stranded and wherein the host cell comprises an expressible gene encoding an RNA polymerase that can transcribe RNA using the single-stranded promoter.

141. The method of claim 140, wherein the expressible gene is operably joined to an inducible promoter.

142. The method of claim 141, wherein the inducible promoter is selected from the group consisting of a bad promoter, a lac promoter, a trp promoter, a tac promoter and a lambda promoter.

143. A method of constructing a nucleic acid library comprising clones of substantially all nucleic acids within a sample by using the method of any one of claims 130 or 140.

144. A method of constructing a nucleic acid library comprising clones of substantially all mRNAs within a sample by using the method of any one of claims 130 or 140.

145. A composition comprising a clone made by using the method of any one of claims 108 - 144.

146. A composition comprising a nucleic acid library made by using the method of claim 129.

147. A host cell comprising a circular DNA molecule made by using the method of claim 108.

148. A circular DNA molecule made by using the method of claim 108.

149. A kit for performing the method of claim 108.

150. A method for detecting an analyte in a sample, the method comprising:

a) obtaining a transcription signaling system comprising a ssDNA comprising:

i) a promoter sequence that binds an RNA polymerase that can transcribe RNA using a single-stranded promoter, and

ii) a signal sequence that is operably joined to the promoter sequence;

b) joining the transcription signaling system to an analyte-binding substance;

c) contacting the analyte-binding substance to which the transcription signaling system is joined with a sample under conditions effective to allow binding of an analyte to the analyte-binding substance and forming a specific binding pair;

d) removing the specific binding pair from the sample;

e) incubating the specific-binding pair with an RNA polymerase that can transcribe RNA using a single-stranded promoter under conditions effective to allow synthesis of a transcription product; and

f) detecting the transcription product.

151. The method of claim 150, wherein the analyte is selected from the group consisting of a biochemical molecule, a biopolymer a protein, a glycoprotein, a lipoprotein, an enzyme, a hormone, a receptor, an antigen, an antibody, a nucleic acid, a DNA, an RNA, a polysaccharide and a lipid.

152). The method of claim 150 wherein the promoter sequence is an N4 vRNAP promoter sequence set forth in SEQ ID NO:16, SEQ ID NO:19, SEQ ID NO:27, SEQ ID NO:28 or SEQ ID NO:29.

153) The method of claim 150 wherein the promoter sequence is a P2 sequence set forth in SEQ ID NO:16 or SEQ ID NO:28.

154) The method of claim 150, wherein the RNA polymerase comprises a region encoding a polypeptide having an amino acid sequence set forth in SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8 or SEQ ID NO:15.

155) The method of claim 150, wherein the RNA polymerase comprises a polypeptide encoded by the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7 or SEQ ID NO:14.

156) The method of claim 150 wherein the RNA polymerase comprises E. coli RNAP and the promoter sequence comprises a single-stranded pseudopromoter for E.coli RNAP.

157) The method of claim 150 wherein the RNA polymerase comprises T7-type RNAP and the promoter sequence comprises a single-stranded pseudopromoter for the T7-type RNAP.

158) The method of claim 150 wherein the RNA polymerase comprises T7 RNAP and the promoter sequence comprises a single-stranded pseudopromoter for T7 RNAP.

159) The method of claim 150, wherein the RNA polymerase comprises T3 RNAP and the promoter sequence comprises a single-stranded pseudopromoter for T3 RNAP.

160) The method of claim 150 wherein the RNA polymerase comprises SP6 RNAP and the promoter sequence comprises a single-stranded pseudopromoter for SP6 RNAP.

161) The method of claim 150 wherein the signal sequence comprises a substrate for Q-beta replicase.

162) The method of claim 150 wherein the signal sequence comprises a sequence that encodes a detectable protein.

163) The method of claim 162 wherein the detectable protein is green fluorescent protein.

164) The method of claim 150 wherein the signal sequence comprises a sequence that is detectable by a probe.

165) The method of claim 164 wherein the sequence that is detectable by a probe comprises a molecular beacon.

166) The method of claim 150, wherein the analyte-binding substance is selected from the group consisting of a nucleic acid, a polynucleotide, an oligonucleotide, a segment of a nucleic acid or polynucleotide, a DNA,an RNA, a molecule comprising both DNA and RNA mononucleosides, modified DNA mononucleosides, a molecule obtained by a method termed "SELEX", a nucleic acid molecule having an affinity for protein molecules, a polynucleotide

molecule having an affinity for protein molecules, an operator, a promoter, an origin of replication, a restriction endonuclease recognition sequence, a ribosomal nucleic acid sequence, a sequence recognized by steroid hormone-receptor complexes, a peptide nucleic acid (PAN), a nucleic acid and a PNA, a molecule prepared by using a combinatorial library of randomized peptide nucleic acids, an oligonucleotide or polynucleotide with a modified backbone that is not an amino acid, a molecule identified by using high throughput screening methods, lectin,a receptor for a hormone, a hormone, and an enzyme inhibitor.

167) The method of claim 150 wherein the binding of step (c) comprises non-covalent bonds.

168) The method of claim 167 wherein the non-covalent bonds comprise hydrogen-bonds.

169) The method of claim 167 wherein the non-covalent bonds comprise hydrophobic interactions.

170) The method of claim 167 wherein the non-covalent bonds comprise van der Waals forces.

171) The method of claim 167 wherein the non-covalent bonds comprise salt bridges.